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SALT AND WATER BALANCE IN TWO MARINE SPIDER CRABS, *LIBINIA EMARGINATA* AND *PUGETTIA PRODUCTA*. III. SOME FACTORS INVOLVED IN SHORT-TERM ADAPTATION TO A DILUTE MEDIUM

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Classical studies (Bethe, 1929; Schlieper, 1929; Bialaszewicz, 1931; Margaria, 1931; Hukuda, 1932; Schwabe, 1933; Nagel, 1934; Huf, 1936; Webb, 1940) have demonstrated that crabs from marine and brackish-water habitats are permeable to salts and water and produce urine at substantial rates. Thus, when an osmoconforming decapod is exposed to a dilute salinity, it is expected that the change in blood concentration will be accompanied by a temporary increase in volume, elimination of salts and water via the urine, and diffusion of salts across the body wall. All of these events are known to occur in osmoconforming crabs; however, there have been few studies in which it has been possible to evaluate and compare the relative importance of these factors. The present study is an attempt to evaluate those factors which are of importance in the short-term salinity adaptation of the osmoconforming spider crabs, *Pugettia producta* (Randall, 1839) and *Libinia emarginata* (Leach, 1815).

Materials and Methods

Specimens of *Pugettia producta* were maintained at the University of California, Berkeley at 10 to 12° C in filtered sea water (SW) (about 1015 milliosmoles) obtained from the Bodega Marine Laboratory, Bodega Bay, California. Specimens of *Libinia emarginata* were maintained at the Marine Biological Laboratory, Woods Hole, Massachusetts at 19 to 21° C in running SW (about 945 milliosmoles).

Methods used in handling samples of blood and urine, and in the determinations of urine production rates, inorganic ion concentrations, and weight changes have been previously described (Cornell, 1979a). Measurements of hydrostatic pressure were made with a Statham model P23BC pressure transducer and the results displayed on a Grass Instruments Co. model 7a polygraph. The pressure transducer was connected via Intramedic P. E. 190 tubing to an 18 gauge hypodermic needle which was inserted through the arthrodial membrane of the first leg base.

Dilutions of SW were made with tap water and initially checked and/or adjusted with a Goldberg refractometer (American Optical Co.), and/or a Perkin-Elmer model 290 atomic absorption spectrophotometer at Berkeley, or a set of hydrometers at Woods Hole. Except for running SW, all media were continuously aerated.

Results

Weight changes and urine production rates in Libinia *and* Pugettia

Weight changes in normal and nephropore-occluded specimens of *Libinia* and *Pugettia* transferred from 100 to 80% SW are presented in Figure 1. In normal

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crabs, maximum weight gains occurred during the first hour after transfer to 80% SW and weight returned to normal by about the tenth hour. *Pugettia* gained a maximum of 1.4% body weight (bw) compared with 0.56% bw for *Libinia*. Nephropore-occluded crabs of both species gained at least 5% bw after 4 hr of exposure to 80% SW.

Empirically, it has been found that an equation of the form $y = Ct\ \exp(-kt)$ provides a good description of average weight changes during the first several hours of exposure to 80% SW. Data may be fitted to this equation by regressing

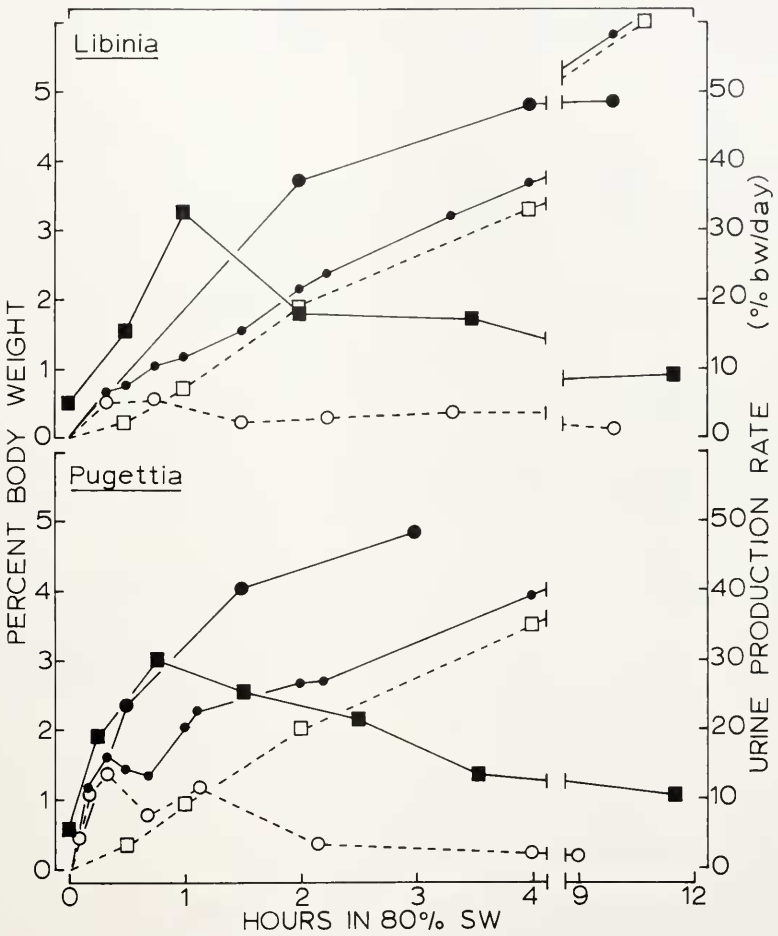

FIGURE 1. Water influx as urine production and volume change in specimens of *Libinia emarginata* and *Pugettia producta* transferred from 100 to 80% sea water. For both species, values for urine production rates (*Libinia*, N = 2; *Pugettia*, N = 9) are indicated by the solid squares and are read from the right ordinates. Values for the data which follow are read from the left ordinates. Open squares indicate the cumulative volume of urine produced from time 0 and are based on the above data. The larger solid circles indicate the weight gains in nephropore-occluded crabs (*Libinia*, N = 7; *Pugettia*, N = 7). Open circles indicate the weight gains in normal crabs (*Libinia*, N = 19; *Pugettia*, N = 10). The smaller solid circles indicate the calculated sum of the cumulative volume of urine, and the weight gain in normal crabs, an estimate of the total amount of water which entered the crab since time 0. Note that there is a discontinuity and a change of scale in the abscissa.

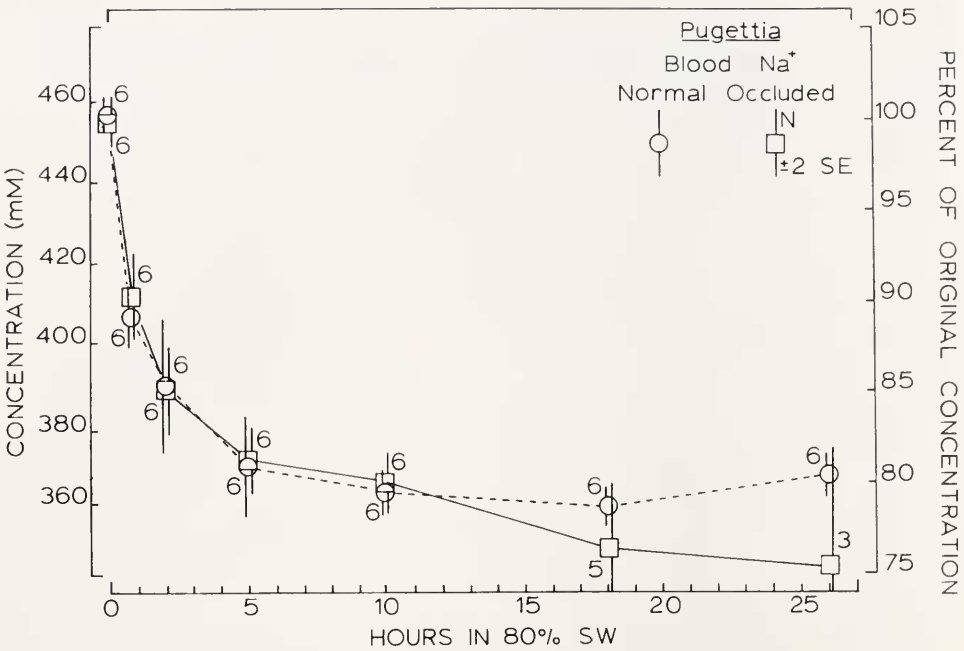

FIGURE 2. Changes in the blood concentration of sodium in normal and nephropore-occluded specimens of *Pugettia producta* transferred from 100 to 80% sea water. Normal (average weight, 104 g) and nephropore-occluded (89 g) crabs are indicated by open circles and squares, respectively. Note that during these experiments, some nephropore-occluded crabs died and that the concentration of the medium changed from 79.5 to 79.0% sea water.

$\log_e [((W_t/W_o) - 1)(1/t)]$ versus t, where t is time, and W_t and W_o are the weights at times t and 0, respectively. In the case of normal crabs, W_t was sometimes less than W_o and therefore the above logarithmic term was not defined. These data were coded by adding 0.01 to the term W_t/W_o and regressed against the square root of time, in order to eliminate the significant deviations from linear regression which coding had introduced. In the case of nephropore-occluded crabs, it was unnecessary to code the data. Analysis of covariance indicates that the intercepts of the regressions for normal and nephropore-occluded specimens of *Pugettia* are significantly greater than those for normal ($P < 0.001$) and nephropore-occluded ($P < 0.05$) specimens of *Libinia,* respectively. No differences between the slopes in either pair of regressions were found. The antilog_e of the intercept is equal to C in the above equation. At time 0 the first derivative is C (even after the data have been coded). Thus, the initial rate of weight gain is greater in specimens of *Pugettia,* and since this is an indication of the rate of water influx, it appears that *Pugettia* is more permeable to water than *Libinia* (see Cornell, 1979b).

In *Pugettia,* the urine production rate increased from about 6 to 30% bw/day during the first hour after transfer to 80% SW. After the first hour, urine production decreased rapidly to about 12% bw/day. The changes in urine production in *Libinia* are similar to those in *Pugettia,* although the data are based on only two animals. Urine production for these two crabs, expressed as the cumulative volume produced from the time of transfer from 100 to 80% SW, is shown in Figure 1.

Changes in hydrostatic pressure in Libinia

The urine production rate should be sensitive to changes in hydrostatic pressure. In 100% SW, the hydrostatic pressure in the ventral sinus was 7.5 ± 2.0 cm H_2O (mean ± SD) in nine specimens of *Libinia* (average weight, 135 g). Normal hydrostatic pressure is partly a function of muscle tone, and sudden increases of about 1.5 cm H_2O could be brought about by visual stimuli, such as sudden hand movements, or turning off the lights. Hydrostatic pressure measurements in three crabs exposed to 80% SW were made. In all crabs, maximum hydrostatic pressure occurred within the first hour of exposure to 80% SW. The average maximum and initial values were 14 and 7.5 cm H_2O, respectively. It took about 7 hr for the hydrostatic pressure to return to its original value.

Changes in the ionic composition of the blood of Pugettia

Sodium concentrations in normal and nephropore-occluded crabs transferred from 100 to 80% SW appear in Figure 2. The data for chloride is very similar to that for sodium. There is little difference between normal and nephropore-occluded crabs with respect to the changes in these two ions. In both groups of crabs the blood concentrations approached 80% of their initial values.

In normal crabs, the blood concentration of magnesium declined slowly (Fig. 3). After 26 hr it was less than 80% of its initial value. This was probably caused by the relative increase in the magnesium excretion rate, the rate being greater than 80% of the rate in 100% SW. In nephropore-occluded crabs, the decline in blood concentration was even slower. In this case it was expected that

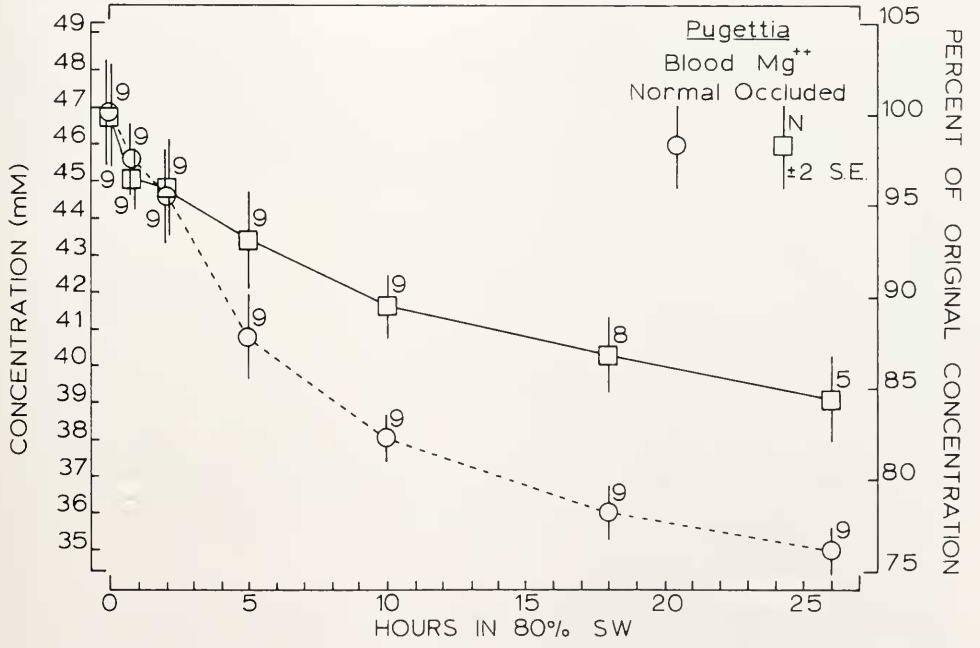

FIGURE 3. Changes in the blood concentration of magnesium in normal and nephropore-occluded specimens of *Pugettia producta* transferred from 100 to 80% sea water. Average weights are 97 and 92 g for normal and nephropore-occluded crabs, respectively. See Figure 2 for other details.

TABLE I

The rates of concentration change of ions in the blood of normal and nephropore-occluded specimens of Pugettia producta *transferred from 100 to 80% sea water. Rates were calculated from the equation* $f'(0) = -kB$ *which is the first derivative at time 0 of* $f(t) = A + B\,exp(-kt)$, *where t is time, A is the concentration at time infinity, B is the change in concentration between time 0 and infinity, and k is a rate constant. A, B, and f(t) were estimated from the data, f(t)being estimated from the group of samples that was taken at the time when about half of the change in concentration had occurred, and k was calculated from these quantities. A relative rate was calculated by dividing the absolute initial rate by the initial concentration,* i.e., $A + B$. *An asterisk denotes that quantity A for calcium and potassium in nephropore-occluded crabs was estimated from that in normal crabs because of the secondary changes in these two ions (see text).*

Condition of nephropores	Ion	f(t) concn. at time t mM	t time hr	A final concn. mM	B concn. change mM	k rate constant 1/hr	$f'(0) = -kB$ initial rate of concn. change mM/hr	$\frac{kB}{A+B}$ relative initial rate of concn. change 1/hr
Normal	Cl^-	471	0.75	423	98	0.952	−93.3	0.179
	Na^+	406	0.75	363	93	1.03	−95.6	0.210
	Mg^{2+}	40.9	5.0	35.6	11.3	0.151	−1.71	0.0365
	Ca^{2+}	9.96	0.75	9.6	1.84	2.18	−4.00	0.350
	K^+	8.34	0.75	8.1	1.97	2.70	−5.32	0.529
Occluded	Cl^-	468	0.75	422	106	1.11	−118	0.224
	Na^+	414	0.75	358	97	0.732	−71.0	0.156
	Mg^{2+}	43.6	5.0	39.5	7.25	0.114	−0.826	0.0177
	Ca^{2+}	9.83	0.75	9.6*	1.96	2.86	−5.60	0.484
	K^+	8.38	0.75	8.1*	2.25	2.69	−6.04	0.585

the concentration would approach that of the medium, *i.e.,* 41.2 mM; but, at 26 hr it was significantly less than this, *i.e.,* 39.5 mM. This could indicate that magnesium is actively removed via extra-renal routes; however, such a conclusion does not seem warranted since by 26 hr a number of nephropore-occluded crabs had died and those remaining were physiologically distressed. It should be noted, however, that salts are concentrated in the gut of *Homarus americanus* when this animal is transferred to a dilute salinity (Dall, 1970).

In normal crabs, calcium declined to a concentration of about 83% of its initial value (Fig. 4). This is consistent with the fact that a considerable amount of calcium is bound to blood proteins (Robertson, 1953; Greenaway, 1976). In nephropore-occluded animals, there were secondary increases in calcium concentrations beginning at about 2 hr after transfer. It seems possible that calcium could be mobilized from the exoskeleton under these conditions. Potassium concentrations in normal animals changed much like those of sodium, except that the change took place at a greater rate. In nephropore-occluded animals there were also secondary increases in potassium, beginning at about 15 hr after transfer, which were correlated with the death of some crabs and which probably resulted from the breakdown of cells.

The means of the concentrations, at 0.75 hr after transfer to 80% SW, expressed as percentages of the initial concentrations, were compared in two single classification analyses of variance. *A priori* tests indicate that in normal crabs the relative concentration of magnesium is greater than ($P < 0.001$), and that for potassium is less than ($P < 0.001$), the relative concentrations of chloride, sodium and calcium. The results are similar in nephropore-occluded crabs, except that

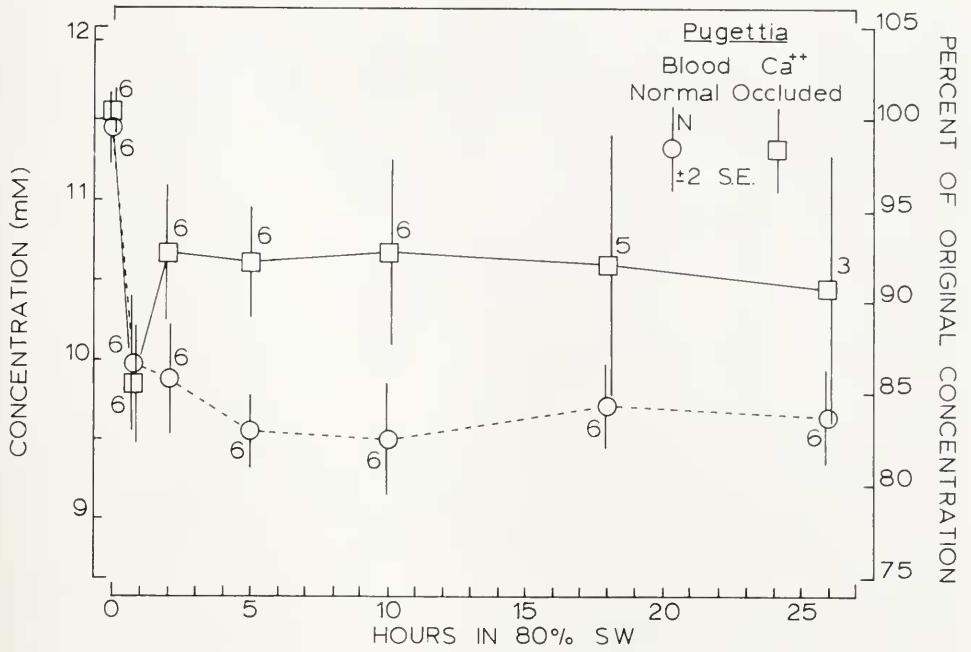

FIGURE 4. Changes in the blood concentration of calcium in normal and nephropore-occluded specimens of *Pugettia producta* transferred from 100 to 80% sea water. See Figure 2 for other details.

there is a difference among chloride, sodium and calcium ($P < 0.05$). Comparison of the relative initial rates of concentration change (Table I), for normal and nephropore-occluded crabs, suggests that the urine is the major route for magnesium loss, but that extra-renal routes are of major importance for the other ions. Some estimates of sodium and magnesium loss via the urine are shown in Table II.

DISCUSSION

Changes in volume and urine production, and diffusion of salts across the body wall are important factors in the short-term adaptation of *Libinia emarginata* and *Pugettia producta* to dilute salinity. Weight gain in these crabs is small when compared to that in some soft bodied animals, *e.g.*, *Phascolosoma* gains 22% bw when placed in 80% SW (Adolph, 1936); however, it serves temporarily to store a large portion of the influx of water (Fig. 1), thus lowering the demand on the antennal gland and reducing the potential hydrostatic pressure. At the time of transfer from 100 to 80% SW, if no weight gain occurred, urine would have to be produced at 25 and 15 times the normal rate in *Pugettia* and *Libinia*, respectively. Between the first and second hours after transfer, urine production accounts for most of the water influx, since urine production increases by a factor of about 5 and the concentration gradient is much reduced.

In both *Libinia* and *Pugettia* the sum of the cumulative volume of urine and the weight gain is less than the weight gain in nephropore-occluded animals (Fig. 1). This difference suggests that it is not possible to predict the change in urine production rate in this situation from the difference between the weight gain in normal and nephropore-occluded animals. This can probably be accounted for by the fact

TABLE II

Time-averaged rates of sodium and magnesium loss in the urine of specimens of Pugettia producta *(average weight, 118 g) transferred from 100 to 80% sea water. Standard deviations (N = 5) are included in parentheses below the means.*

Time period hr	Na^+ micromole/ (g·hr)	Mg^{2+} micromole/ (g·hr)	Urine flow rate μl/(g·hr)
0–2	3.97 (2.08)	0.539 (0.266)	8.97 (4.89)
2–5	2.19 (1.04)	0.289 (0.136)	5.30 (2.48)
5–10	2.03 (0.511)	0.281 (0.080)	5.28 (1.20)

that not only does the urine eliminate water, but since it is isosmotic to the blood, it also eliminates considerable quantities of salts. In the absence of urine production, these salts, particularly the less permeant ones, tend to remain in the blood where their osmotic activity causes a further increase in volume.

In *Libinia*, the increase in urine production can be correlated with an increase in hydrostatic pressure. However, urine production increased by a factor of about 5 while the hydrostatic pressure did not quite double. Unfortunately, it is not known how the hydrostatic pressure across the coelomosac is affected by the increase in hydrostatic pressure in the thoracic sinus. In *Carcinus*, the increase in hydrostatic pressure does not seem to totally account for the increase in urine production (Norfolk, 1978). Norfolk suggests that *Carcinus* might increase urine production by "anticipating" the change in internal conditions from the change in the external medium. It is not clear whether this is the case in *Libinia* and *Pugettia;* however, changes in hydrostatic pressure in *Libinia* do not seem to explain why urine production did not soon return to normal, since hydrostatic pressure did. There is evidence for the presence of diuretic hormones among the decapods (see Kamemoto and Tullis, 1972), and their presence in *Libinia* and *Pugettia* might explain why urine production did not immediately return to normal.

During the first two hours of exposure to 80% SW, direct estimates of the time-averaged rates of renal loss of sodium and magnesium are 4.0 and 0.54 micromole/(g·hr), respectively (Table II). These estimates are probably high because of the influence of the initial amounts of ions contained in the bladders. Independent estimates can be made by integrating the product of the blood concentration and the urine production rates represented by $f(t) = A + B\exp(-kt)$ and $g(t) = D + Ct\exp(-jt)$, respectively:

$$\int f(t)g(t)dt = ADt + \frac{BDe^{-kt}}{-k} - \frac{(jt+1)ACe^{-jt}}{j^2} - \frac{((j+k)t+1)BCE^{-(j+k)t}}{(j+k)^2}.$$

Definitions and values for A, B and k are found in Table I. D is the rate of urine production for crabs in 100% SW and the term $Ct\exp(-jt)$ was arbitrarily chosen to represent the increase in urine production of crabs exposed to 80% SW. When expressed in units of ml/(g·hr), values of D, C and j are 0.0025, 0.024 and 0.98, respectively, for the crabs in Table II.

Evaluating this integral for sodium and magnesium from 0 to 2 hr and dividing the results by 2 hr, so that they are expressed in a form comparable to that in Table II, indicates that sodium and magnesium were filtered from the blood at 3.9

and 0.44 micromole/(g·hr), respectively. It is also necessary to account for the rate at which magnesium is actively concentrated in the urine. In 100% SW, magnesium is 24 mM greater in the urine than in the blood, while the urine production rate is 0.0025 ml/(g·hr) (Cornell, 1979a). Thus, magnesium is concentrated in the urine at 0.06 micromole/(g·hr) and it leaves via the urine, during the first 2 hr, at 0.44 + 0.06 = 0.50 micromole/(g·hr). These estimates are not much lower than those obtained by direct measurement which suggests that the bladder volumes were not particularly large in these animals.

Total rates of ion loss were not measured, but some estimates can be made. It will be assumed that the ion space is constant, since the maximum volume change in normal specimens of *Pugettia* is small (about 5% of the ion space). Twenty-five percent bw can be assumed to be a minimum estimate for the ion space, since this is the inulin space in intermolt specimens of *Pugettia* (Born, 1970). Thirty-five percent bw can be considered a reasonable upper limit. A few examples from studies on *Carcinus maenas*, which is approximately isotonic to the medium when in 100% SW, will suffice: when the total body chloride was assumed to be in the blood, its volume was calculated as 36% bw (Webb, 1940); the calcium space is 25% bw (Greenaway, 1976); the inulin space is 19% bw (Binns, 1969). No doubt some inorganic ions of intracellular origin enter the blood during exposure to 80% SW, but the amounts are small. Changes in free amino acids account for the major change in intracellular osmotic concentration when *Libinia* is transferred to 50% SW (Gilles, 1970). Freel (1978) has shown that the intracellular concentrations of potassium, and calcium plus magnesium, in the muscles of a number of decapods, are independent of extracellular concentration. However, intracellular sodium and chloride concentrations were found to be a function of the extracellular concentration and thus some sodium of intracellular origin probably entered the blood. But, since the intracellular sodium concentration is low (about 20 mM), the following results can be affected by no more than 5%.

The time-averaged rate of ion loss can be estimated as $(1/t)VB(1 - \exp(kt))$; where t is time, V is the weight-specific ion space (*e.g.*, 0.35 ml/g), B is the change in ion space concentration between times 0 and infinity, and k is a rate constant. Values for B and k are found in Table I. For normal animals, assuming an ion space of 35% bw, the rates of sodium and magnesium loss are 14 and 0.51 micromole/(g·hr), respectively. If 25% bw is assumed, these rates are 10 and 0.36 micromole/g·hr), respectively. Thus, the renal rate of sodium loss is less than 40% of the total, while the renal rate of magnesium loss must nearly equal the total rate of loss. Most studies of osmoregulating decapods have shown that renal salt loss, that is of sodium or chloride, is less than 25% of the total (*Eriocheir*, Krogh, 1938; *Carcinus*, Shaw, 1961; *Astacus*, Bryan, 1960; *Rithropanopeus*, Smith, 1967; *Potamon*, Harris, 1975; *Paranephrops*, Wong and Freeman, 1976; *Uca*, Baldwin and Kirschner, 1976a, b; *Callinectes*, Cameron 1978).

One question of interest is what happens when animals are transferred to a more concentrated medium. In this case, urine production would be of little importance unless produced at negative rates. When *Pugettia* was transferred to 100% SW, after acclimation to 80% SW for five days, there were no differences between the weight changes in normal and nephropore-occluded crabs. However, normal specimens of *Pugettia* and *Libinia* show larger absolute weight changes when transferred back to 100% SW (1.8 and 0.75% bw, respectively) than when transferred to 80% SW (1.4 and 0.56% bw, respectively). These data suggest that extra-renal routes are of primary importance and reflect the asymmetry of

the transfers caused by the presence or absence of the renal route of ion movement. Thus in *Libinia* and *Pugettia,* volume change and diffusion of salts across the body wall are factors of general importance whenever these animals are exposed to a change in salinity. Urine production is an important factor when animals are exposed to a dilute salinity, but seems to be of no importance during an exposure to a concentrated salinity.

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Summary

1. *Libinia emarginata* and *Pugettia producta* respond to 80% sea water (SW) in similar ways. In both species, during the first hour of exposure to 80% SW an increase in volume, 0.56 and 1.4% body weight (bw) for *Libinia* and *Pugettia* respectively, accommodates the major portion of the influx of water.

2. Urine production increases from about 6 to 30% bw/day in both species during the first hour of exposure to 80% SW. Elimination of salts and water via the isosmotic urine helps to decrease the potential for an increase in hydrostatic pressure. The hydrostatic pressure in *Libinia* changes from 7.5 to 14 cm H_2O during the first hour of exposure to 80% SW.

3. In *Pugettia,* most of the ions in the blood decrease at a rate proportional to the concentration difference between the blood and the medium. The ordered rates of concentration changes are as follows: potassium > calcium > sodium $\simeq$ chloride >> magnesium. Blocking the nephropores has a greater effect on the rate of magnesium concentration change than on the other ions. With the exception of magnesium, all other ions which were measured are lost from the blood mainly via extra-renal routes.

4. During the second hour of exposure to 80% SW, the volume of both species declines, probably as a result of the increase in urine production and the reduction in the rate of water influx caused by the reduction in the concentration gradient. By contrast, the volume of nephropore-occluded crabs of both species continues to increase, reaching at least 5% bw after 4 hr of exposure to 80% SW.

5. When transferred from 80 to 100% SW, specimens of *Libinia* and *Pugettia* lose 0.75 and 1.8% bw, respectively. Blocking the nephropores has no effect in this case and the gain in salts must take place entirely across the body surface, since urine flow is unidirectional.

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